Hepatoprotective effects of systemic ER activation
Single cell analysis

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19 September, 2022

```r
# source and library import
source('code/00_helper_functions.R')
library(tidyverse)
library(Seurat)
library(pagoda2)
library(patchwork)
```

```r
# color palettes
colPals <- list()
colPals$conditions <- setNames(c('#E98BB6', '#B02262', '#7F9AD7', '#2A2F72', '#7DC7D1', '#339ACD', '#35(
                                 c('CDf', 'HFDf', 'CDm', 'HFDm', 'DPN', 'DIP', 'E2', 'PPT'))
colPals$RdBu <- rev(RColorBrewer::brewer.pal(n=11, name = 'RdBu'))
colPals$UpDown <- setNames(colPals$RdBu[c(10,2)],
                           c('up', 'down'))
colPals$clusters <- setNames(c('#A9D265', '#82506D', '#FA9F1C', '#676A6E'),
                             c('1', '2', '3', '4'))
colPals$celltypes <- setNames(c('#B4272F', '#E5462D', '#FFD1D1', '#F4E54C', '#FBAA3E', '#AA654E', '#B58I
                                '#B3177E', '#CAC1DD', '#67227D','#36B449', '#82C349', '#A9D265', '#19941
                              c('Cholangiocytes', 'Endothelial cells', 'HsPCs', 'Stromal cells', 'Hepato
                                'Kupffer cells', 'Monocytes & Monocyte-derived cells', 'T cells', 'NK ce
                                'ILC1s', 'B cells', 'cDC1s', 'cDC2s', 'Mig. cDCs', 'pDCs', 'Basophils',
colPals$inferno <- c('#FCFFA4', '#FCA50A', '#DD513A', '#932667', '#420A68', '#000004')
```

Load data

```r
# Liver Cell Atlas data from Guilliams et al. 2022 (https://www.livercellatlas.org/download.php)
liver <- readRDS(file = 'data/livercellatlas_feb2022.rds')

# Liver Cell Atlas sample metadata
liver_meta <- read.table(
  file = 'data/livercellatlas_feb2022_sample_annotation.tsv',
  stringsAsFactors = FALSE,
  sep = '\t',
  header = TRUE)

# gene sets
DEG_sets <- readRDS('results/bulkRNAseq_mmus_DEG_sets.rds')
pathway_sets <- readRDS('results/bulkRNAseq_mmus_GSEA_reactome_cluster_sets.rds')
```
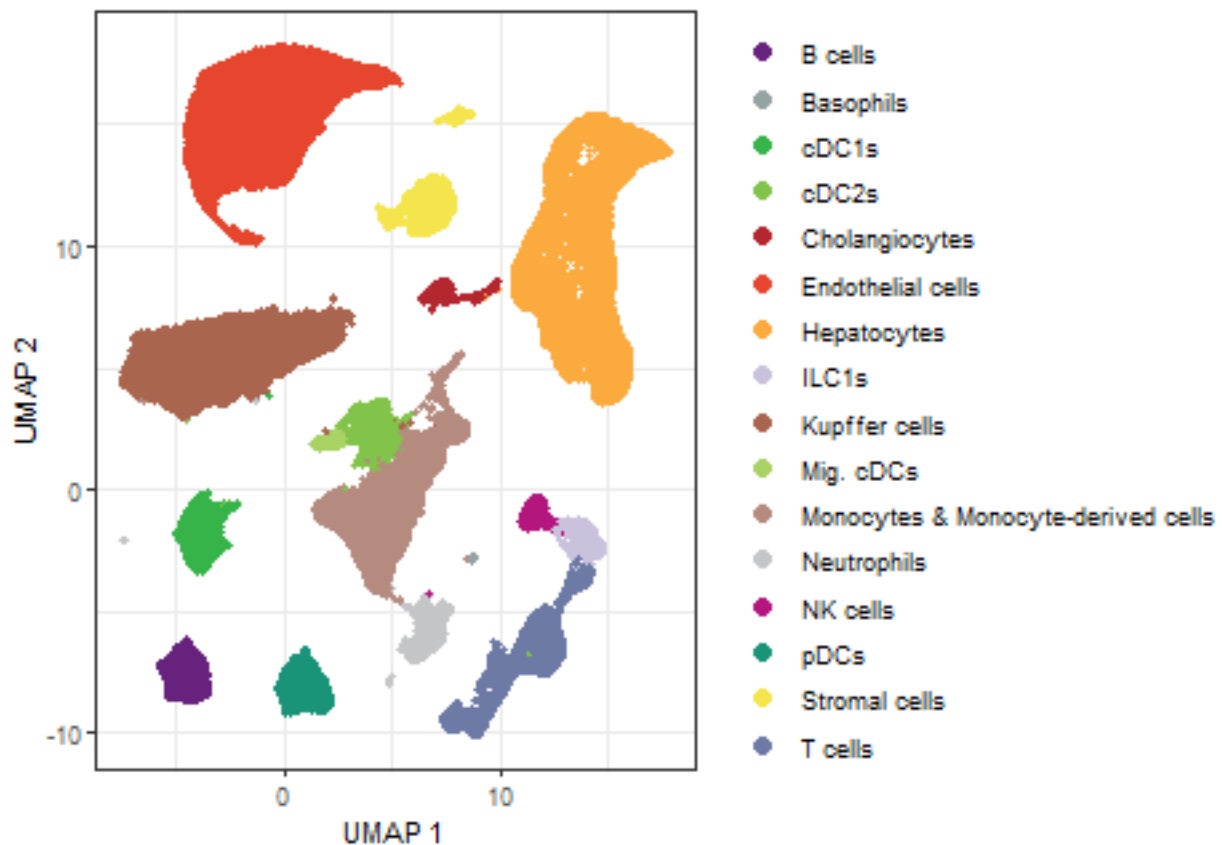
Mouse single-cell map

```r
df <- liver$mouseStSt$All@meta.data %>%
  dplyr::left_join(liver_meta, by = 'sample') %>%
  dplyr::filter(gender == 'male' & annot != 'HsPCs') %>%
  dplyr::mutate(annot = replace(annot, annot == 'Fibroblasts', 'Stromal cells'))

ggplot(df, aes(x=UMAP_1, y=UMAP_2, color=annot, fill=annot, label=annot)) +
  geom_point(shape=21, size=1) +
  scale_fill_manual(values = colPals$celltypes) +
  scale_color_manual(values = colPals$celltypes) +
  xlab("UMAP 1") + ylab("UMAP 2") +
  theme_bw() +
  guides(colour = guide_legend(override.aes = list(size=3))) +
  theme(legend.title = element_blank())
```


Cell type composition

```r
df <- liver$mouseStSt$All@meta.data %>%
  dplyr::left_join(liver_meta, by = 'sample') %>%
  dplyr::filter(gender == 'male' & annot != 'HsPCs') %>%
  dplyr::mutate(annot = replace(annot, annot == 'Fibroblasts', 'Stromal cells')) %>%
  dplyr::filter(digest == 'inVivo') %>%
  dplyr::group_by(annot) %>%
```

```r
    dplyr::summarize(mouseStSt_n = dplyr::n()) %>%
    dplyr::mutate(mouseStSt_freq = round(mouseStSt_n / sum(mouseStSt_n) * 100, 2))

df2 <- liver$mouseNAFLD$All@meta.data %>%
  dplyr::left_join(liver_meta, by = 'sample') %>%
  dplyr::filter(gender == 'male' & annot != 'HsPCs') %>%
  dplyr::filter(digest == 'inVivo') %>%
  dplyr::mutate(annot = replace(annot, annot == 'KCs', 'Kupffer cells')) %>%
  dplyr::mutate(annot = replace(annot, annot == 'Mono/mono-derived cells', 'Monocytes & Monocyte-derive
  dplyr::mutate(annot = replace(annot, annot == 'Mig cDCs', 'Mig. cDCs')) %>%
  dplyr::group_by(annot) %>%
  dplyr::summarize(mouseNAFLD_n = dplyr::n()) %>%
  dplyr::mutate(mouseNAFLD_freq = round(mouseNAFLD_n / sum(mouseNAFLD_n) * 100, 2))

df <- dplyr::full_join(df, df2, by = 'annot') %>%
  replace(is.na(.), 0) %>%
  dplyr::mutate(mouseStSt_minus_NAFLD_freq = mouseStSt_freq - mouseNAFLD_freq) %>%
  tidyr::pivot_longer(cols = c(mouseStSt_freq, mouseNAFLD_freq), names_to = 'cond', values_to = 'freq')
  dplyr::mutate(annot = factor(annot, levels = rev(names(colPals$celltypes))),
               cond = factor(cond, levels = c('mouseStSt_freq','mouseNAFLD_freq')))

ggplot(df, aes(x=freq, y=annot, fill=cond, label=freq)) +
  geom_bar(color="black", size=0.5, width=0.8, position="stack", stat="identity") +
  geom_text(size=3, hjust=-0.2) +
  scale_fill_manual(values = unname(colPals$conditions[c('CDm', 'HFDm')])) +
  scale_x_continuous(expand = expansion(mult = c(.0, .1)),
                     limit = c(0, 50),
                     breaks = c(0, 25, 50)) +
  facet_wrap(~cond) +
  xlab('Abundance (%)') +
  theme_bw() +
  theme(strip.background = element_blank())
```
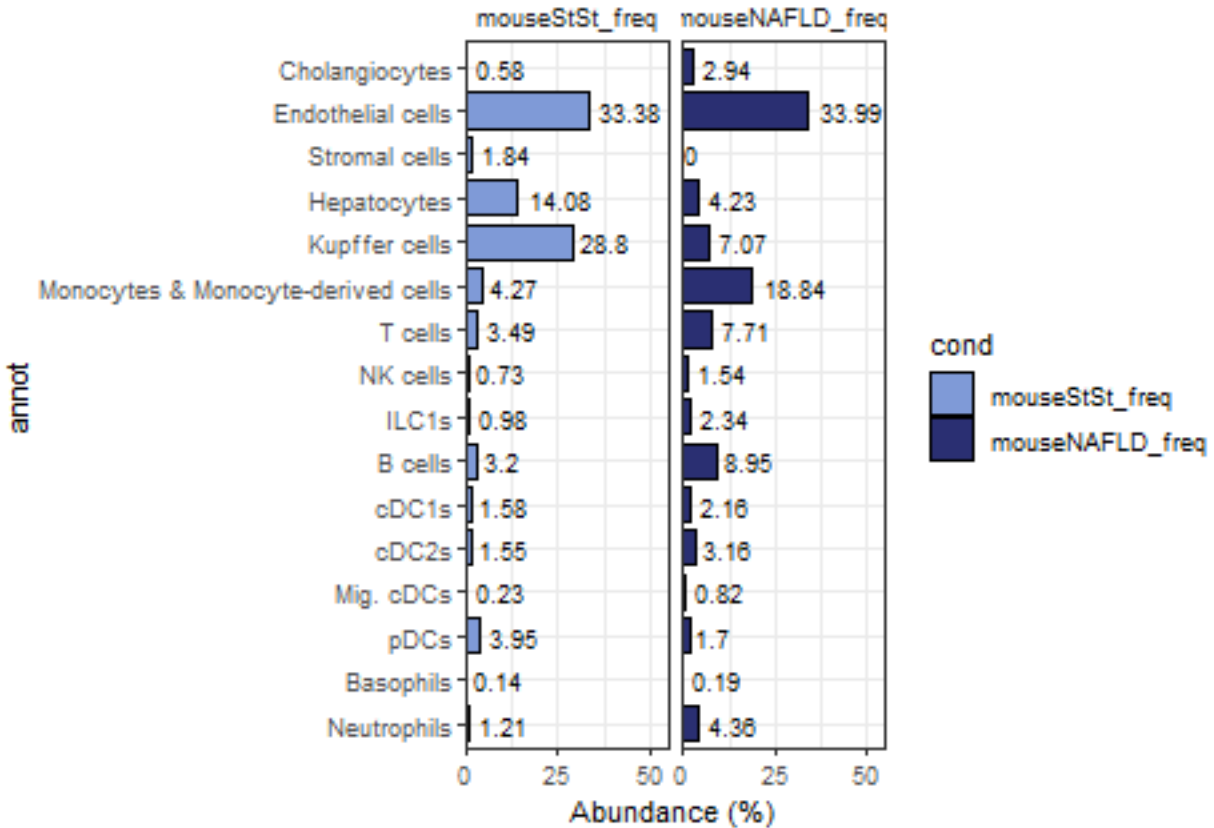
```
# Cell type frequency change (%CD - %HFD)
df2 <- df %>%
  dplyr::select(annot, mouseStSt_minus_NAFLD_freq) %>%
  dplyr::filter(!duplicated(.)) %>%
  dplyr::mutate(change=ifelse(mouseStSt_minus_NAFLD_freq>0, 'up', 'down'))

ggplot(df2, aes(x=mouseStSt_minus_NAFLD_freq, y=annot, fill=change)) +
  geom_bar(color="black", size=0.5, width=0.8, position="stack", stat="identity") +
  scale_fill_manual(values = c("#2166AC","#B2182B")) +
  scale_x_continuous(expand = expansion(mult = c(.0, .1)),
                     limit = c(-25, 25),
                     breaks = c(-20, 0, 20)) +
  xlab('Change (%)') +
  theme_bw() +
  theme(legend.position = 'none')
```

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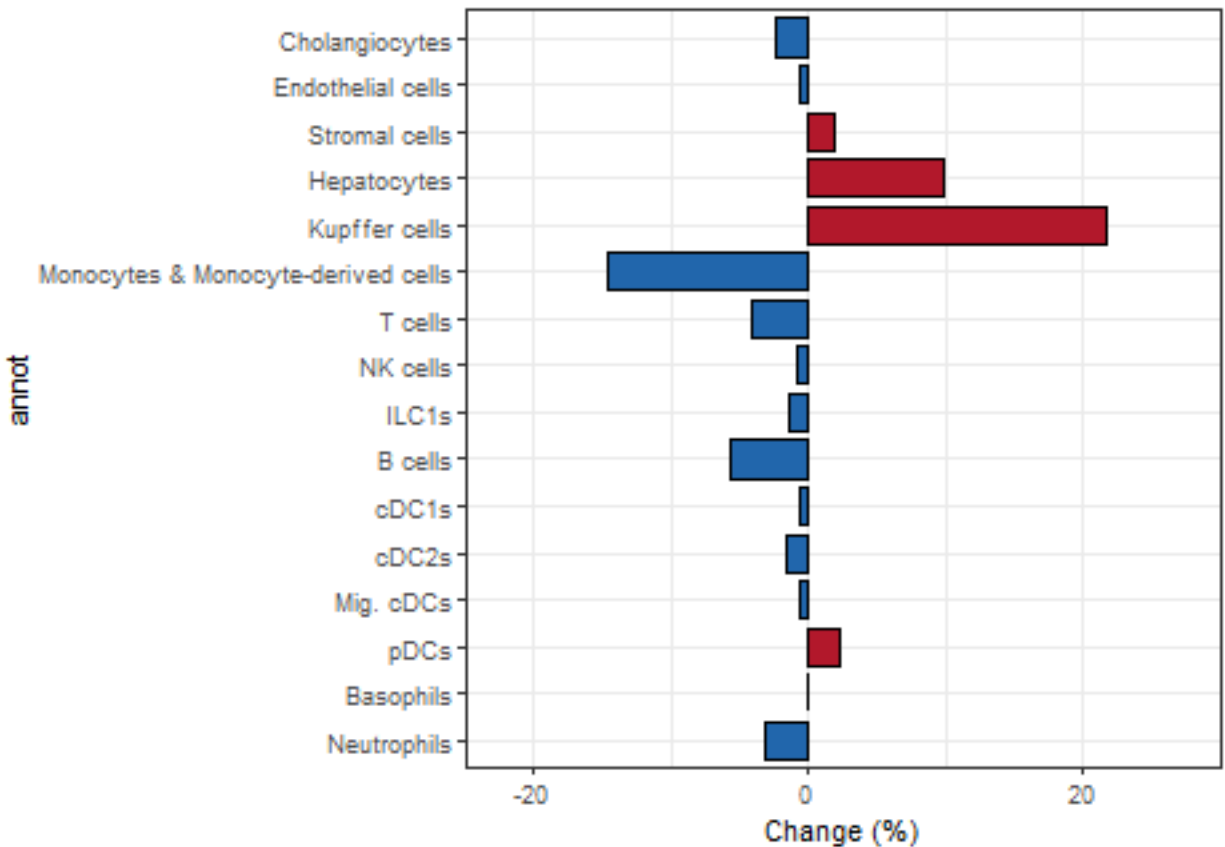
Cell type specificity of ER activation signatures

```r
# subsample single-cell data to 5000 cells per cell type for enrichment analysis
dat <- liver$mouseStSt$All
subs <- dat@meta.data %>%
  tibble::rownames_to_column(var = 'cellid') %>%
  dplyr::left_join(liver_meta, by = 'sample') %>%
  dplyr::filter(gender == 'male' & annot != 'HsPCs') %>%
  dplyr::group_by(annot) %>%
  dplyr::slice_sample(n=5000)
dat <- subset(dat, cells = subs$cellid)

res <- getPAS(x = dat,
              gene.sets = DEG_sets$gene_symbols,
              npcs = 100)

p <- lapply(names(DEG_sets$gene_symbols), function(set) {
  local({
    df <- data.frame(UMAP_1 = res$UMAP_1,
                     UMAP_2 = res$UMAP_2)
    df$score <- res[, set]
    p1 <- ggplot(df, aes(x=UMAP_1, y=UMAP_2, color=score)) +
      geom_point(shape=19, size=1) +
      scale_color_gradientn(colours = colPals$inferno) +
      theme_void() +
```
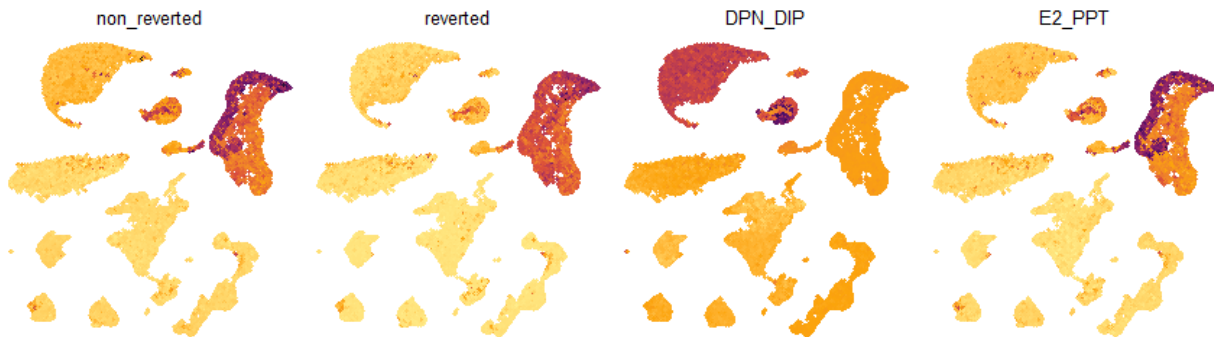
```
      theme(legend.position = 'none',
            plot.title = element_text(hjust = 0.5)) +
      ggtitle(set)
  })
})

patchwork::wrap_plots(p, nrow=1, ncol=4, byrow=T, guides = 'collect')
```


Conservation of cell type specificities in primates

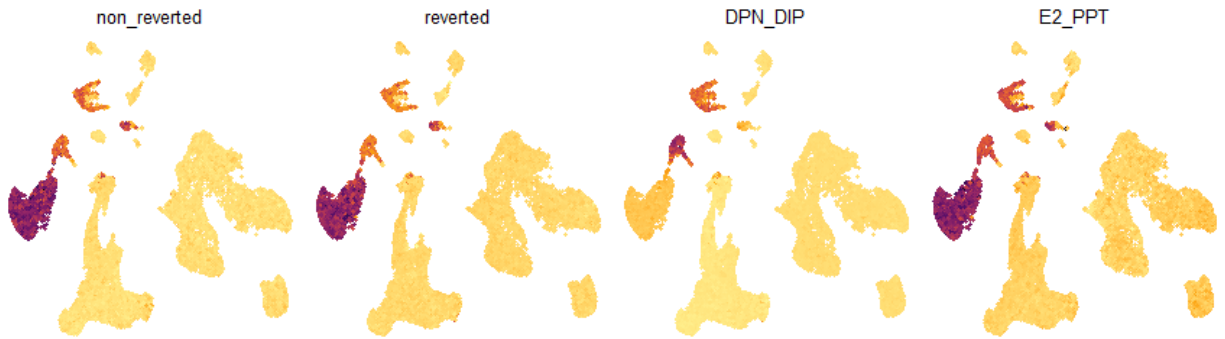### Human

```
# subsample single-cell data to 5000 cells per cell type for enrichment analysis
dat <- liver$human$All
subs <- dat@meta.data %>%
  tibble::rownames_to_column(var = 'cellid') %>%
  dplyr::left_join(liver_meta, by = 'sample') %>%
  dplyr::filter(gender == 'male') %>%
  dplyr::group_by(annot) %>%
  dplyr::slice_sample(n=5000)
dat <- subset(dat, cells = subs$cellid)

res <- getPAS(x = dat,
              gene.sets = lapply(DEG_sets$gene_symbols, toupper),
              npcs = 100)

p <- lapply(names(DEG_sets$gene_symbols), function(set) {
  local({
    df <- data.frame(UMAP_1 = res$UMAP_1,
                     UMAP_2 = res$UMAP_2)
    df$score <- res[, set]
    p1 <- ggplot(df, aes(x=UMAP_1, y=UMAP_2, color=score)) +
      geom_point(shape=19, size=1) +
      scale_color_gradientn(colours = colPals$inferno) +
      theme_void() +
      theme(legend.position = 'none',
            plot.title = element_text(hjust = 0.5)) +
      ggtitle(set)
  })
})
```

```
patchwork::wrap_plots(p, nrow=1, ncol=4, byrow=T, guides = 'collect')
```


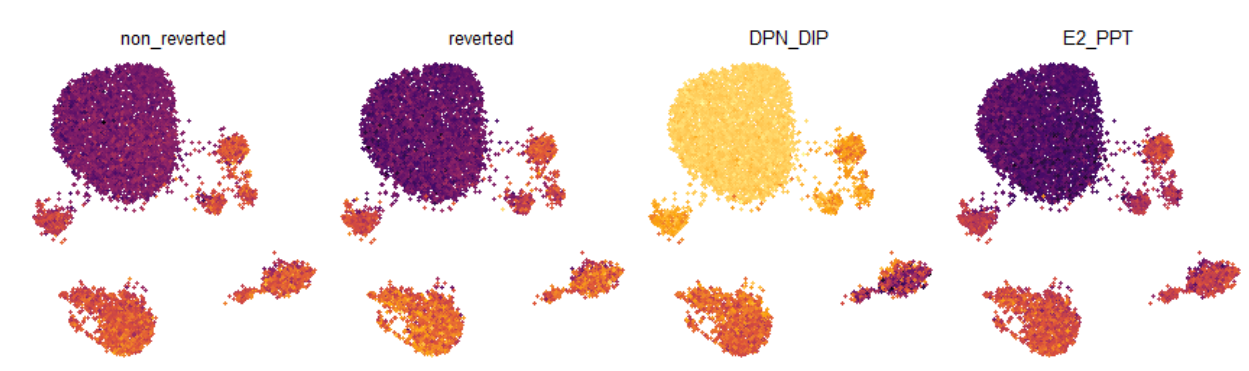## Macaque

```
# subsample single-cell data to 5000 cells per cell type for enrichment analysis
dat <- liver$multisp$monkey
subs <- dat@meta.data %>%
  tibble::rownames_to_column(var = 'cellid') %>%
  dplyr::left_join(liver_meta, by = 'sample') %>%
  dplyr::filter(gender == 'male') %>%
  dplyr::group_by(annot) %>%
  dplyr::slice_sample(n=5000)
dat <- subset(dat, cells = subs$cellid)

res <- getPAS(x = dat,
              gene.sets = lapply(DEG_sets$gene_symbols, toupper),
              npcs = 100)

p <- lapply(names(DEG_sets$gene_symbols), function(set) {
  local({
    df <- data.frame(UMAP_1 = res$UMAP_1,
                     UMAP_2 = res$UMAP_2)
    df$score <- res[, set]
    p1 <- ggplot(df, aes(x=UMAP_1, y=UMAP_2, color=score)) +
      geom_point(shape=19, size=1) +
      scale_color_gradientn(colours = colPals$inferno) +
      theme_void() +
      theme(legend.position = 'none',
            plot.title = element_text(hjust = 0.5)) +
      ggtitle(set)
  })
})

patchwork::wrap_plots(p, nrow=1, ncol=4, byrow=T, guides = 'collect')
```
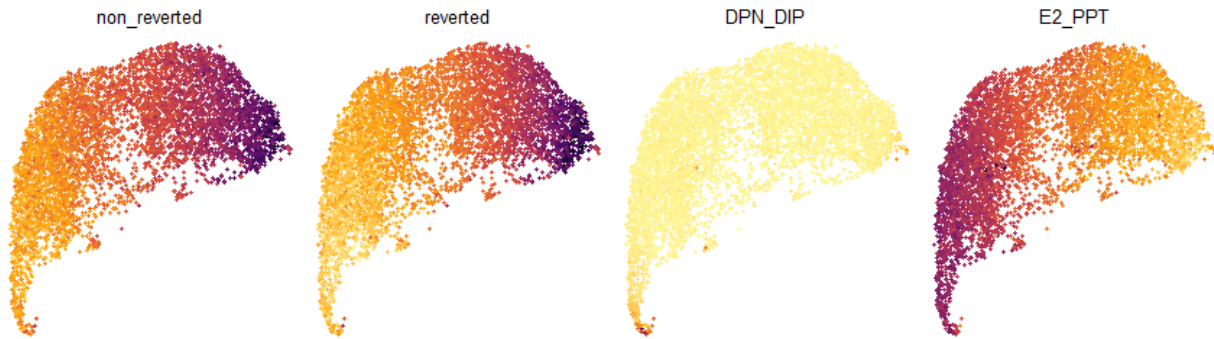
Liver zoonation of ER activation signatures (spatial transcriptomics)

```r
# subsample single-cell data to 5000 cells per cell type for enrichment analysis
dat <- liver$mouseStSt$Visium
subs <- dat@meta.data %>%
  tibble::rownames_to_column(var = 'cellid') %>%
  dplyr::left_join(liver_meta, by = 'sample') %>%
  dplyr::filter(gender == 'male') %>%
  dplyr::group_by(zonationGroup) %>%
  dplyr::slice_sample(n=5000)
dat <- subset(dat, cells = subs$cellid)

res <- getPAS(x = dat,
              gene.sets = DEG_sets$gene_symbols,
              npcs = 100)

p <- lapply(names(DEG_sets$gene_symbols), function(set) {
  local({
    df <- data.frame(UMAP_1 = res$UMAP_1,
                     UMAP_2 = res$UMAP_2)
    df$score <- res[, set]
    p1 <- ggplot(df, aes(x=UMAP_1, y=UMAP_2, color=score)) +
      geom_point(shape=19, size=1) +
      scale_color_gradientn(colours = colPals$inferno) +
      theme_void() +
      theme(legend.position = 'none',
            plot.title = element_text(hjust = 0.5)) +
      ggtitle(set)
  })
})

patchwork::wrap_plots(p, nrow=1, ncol=4, byrow=T, guides = 'collect')
```

Pathway enrichment across cell types

Steady State
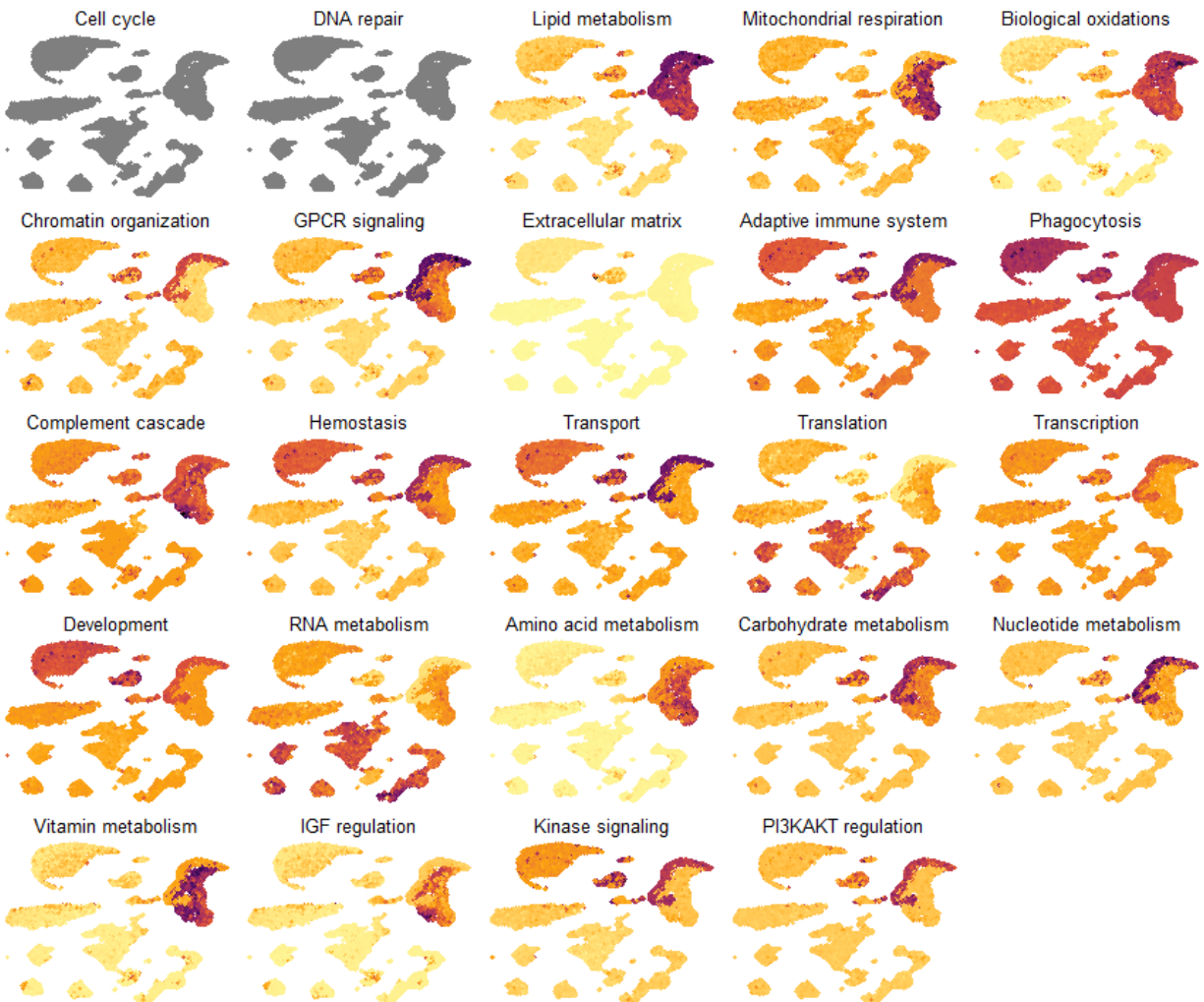
```r
# subsample single-cell data to 5000 cells per cell type for enrichment analysis
dat <- liver$mouseStSt$All
subs <- dat@meta.data %>%
  tibble::rownames_to_column(var = 'cellid') %>%
  dplyr::left_join(liver_meta, by = 'sample') %>%
  dplyr::filter(gender == 'male' & annot != 'HsPCs') %>%
  dplyr::group_by(annot) %>%
  dplyr::slice_sample(n=5000)
dat <- subset(dat, cells = subs$cellid)

res <- getPAS(x = dat,
              gene.sets = pathway_sets,
              npcs = 100)

df <- res %>%
  dplyr::mutate(annot = replace(annot, annot == 'Fibroblasts', 'Stromal cells')) %>%
  dplyr::group_by(annot) %>%
  dplyr::summarize(dplyr::across(`Adaptive immune system`:`Vitamin metabolism`, mean)) %>%
  dplyr::mutate(across(`Adaptive immune system`:`Vitamin metabolism`, ~ (.-min(.)) / (max(.)-min(.)) ))
  tidyr::pivot_longer(cols = `Adaptive immune system`:`Vitamin metabolism`, names_to = 'pathway', values
  dplyr::mutate(annot = factor(annot, levels = rev(names(colPals$celltypes))),
               pathway = factor(pathway, levels = names(pathway_sets)))

p <- lapply(names(pathway_sets), function(set) {
  local({
    df <- data.frame(UMAP_1 = res$UMAP_1,
                     UMAP_2 = res$UMAP_2)
    df$score <- res[, set]
    p1 <- ggplot(df, aes(x=UMAP_1, y=UMAP_2, color=score)) +
      geom_point(shape=19, size=1) +
      scale_color_gradientn(colours = colPals$inferno) +
      theme_void() +
      theme(legend.position = 'none',
            plot.title = element_text(hjust = 0.5)) +
      ggtitle(set)
  })
})
```

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```
patchwork::wrap_plots(p, ncol=5, byrow=T, guides = 'collect')
```


NAFLD

```
# subsample single-cell data to 5000 cells per cell type for enrichment analysis
dat <- liver$mouseNAFLD$All
subs <- dat@meta.data %>%
  tibble::rownames_to_column(var = 'cellid') %>%
  dplyr::left_join(liver_meta, by = 'sample') %>%
  dplyr::filter(gender == 'male' & annot != 'HsPCs') %>%
  dplyr::group_by(annot) %>%
  dplyr::slice_sample(n=5000)
dat <- subset(dat, cells = subs$cellid)


res <- getPAS(x = dat,
              gene.sets = pathway_sets,
              npcs = 100)


p <- lapply(names(pathway_sets), function(set) {
```
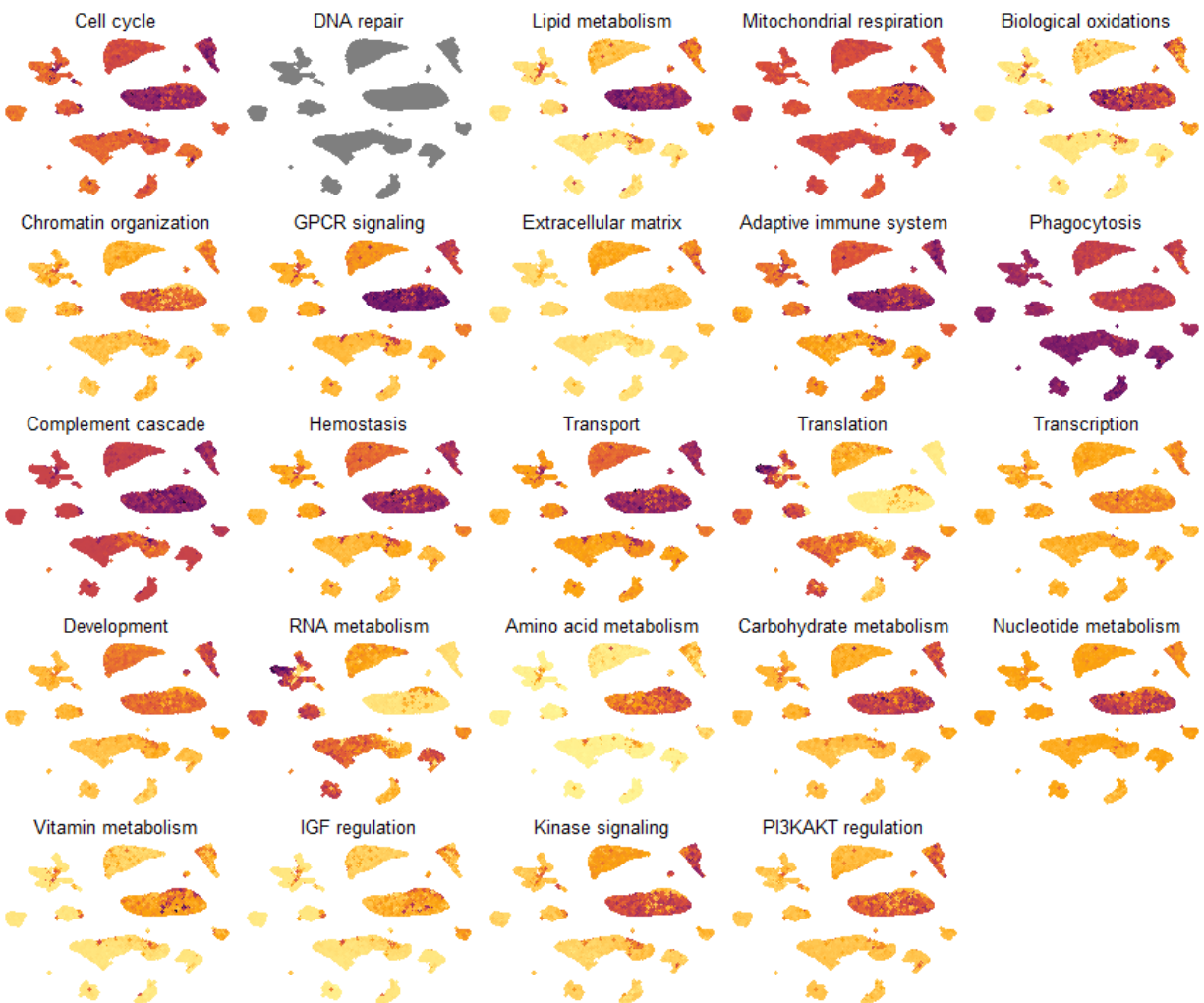
```
local({
  df <- data.frame(UMAP_1 = res$UMAP_1,
                   UMAP_2 = res$UMAP_2)
  df$score <- res[, set]
  p1 <- ggplot(df, aes(x=UMAP_1, y=UMAP_2, color=score)) +
    geom_point(shape=19, size=1) +
    scale_color_gradientn(colours = colPals$inferno) +
    theme_void() +
    theme(legend.position = 'none',
          plot.title = element_text(hjust = 0.5)) +
    ggtitle(set)
})
})

patchwork::wrap_plots(p, ncol=5, byrow=T, guides = 'collect')
```


## Comparison of steady state vs NAFLD

```
df2 <- res %>%
  dplyr::mutate(annot = replace(annot, annot == 'KCs', 'Kupffer cells')) %>%
```

```r
  dplyr::mutate(annot = replace(annot, annot == 'Mono/mono-derived cells', 'Monocytes & Monocyte-derived
  dplyr::mutate(annot = replace(annot, annot == 'Mig cDCs', 'Mig. cDCs')) %>%
  dplyr::group_by(annot) %>%
  dplyr::summarize(dplyr::across(`Adaptive immune system`:`Vitamin metabolism`, mean)) %>%
  dplyr::mutate(dplyr::across(`Adaptive immune system`:`Vitamin metabolism`, ~ (.-min(.)) / (max(.)-min
  tidyr::pivot_longer(cols = `Adaptive immune system`:`Vitamin metabolism`, names_to = 'pathway', values
  dplyr::mutate(cond = 'HFD')

df3 <- df %>%
  dplyr::mutate(cond = 'CD') %>%
  dplyr::bind_rows(df2) %>%
  dplyr::mutate(cond = factor(cond, levels = c('CD','HFD')),
                annot = factor(annot, levels = names(colPals$celltypes)),
                pathway = factor(pathway, levels = rev(names(pathway_sets))))

ggplot(df3, aes(x=annot, y=pathway, size=score, color=score)) +
  geom_point() +
  scale_color_gradientn(colours = colPals$inferno) +
  scale_size(range = c(1,8))+
  facet_wrap(~cond) +
  theme_bw() +
  theme(strip.background = element_blank(),
        axis.text.x = element_text(angle = 45, hjust = 1, vjust = 1),
        axis.text.y = element_text(hjust = 1, vjust = 0.3))
```
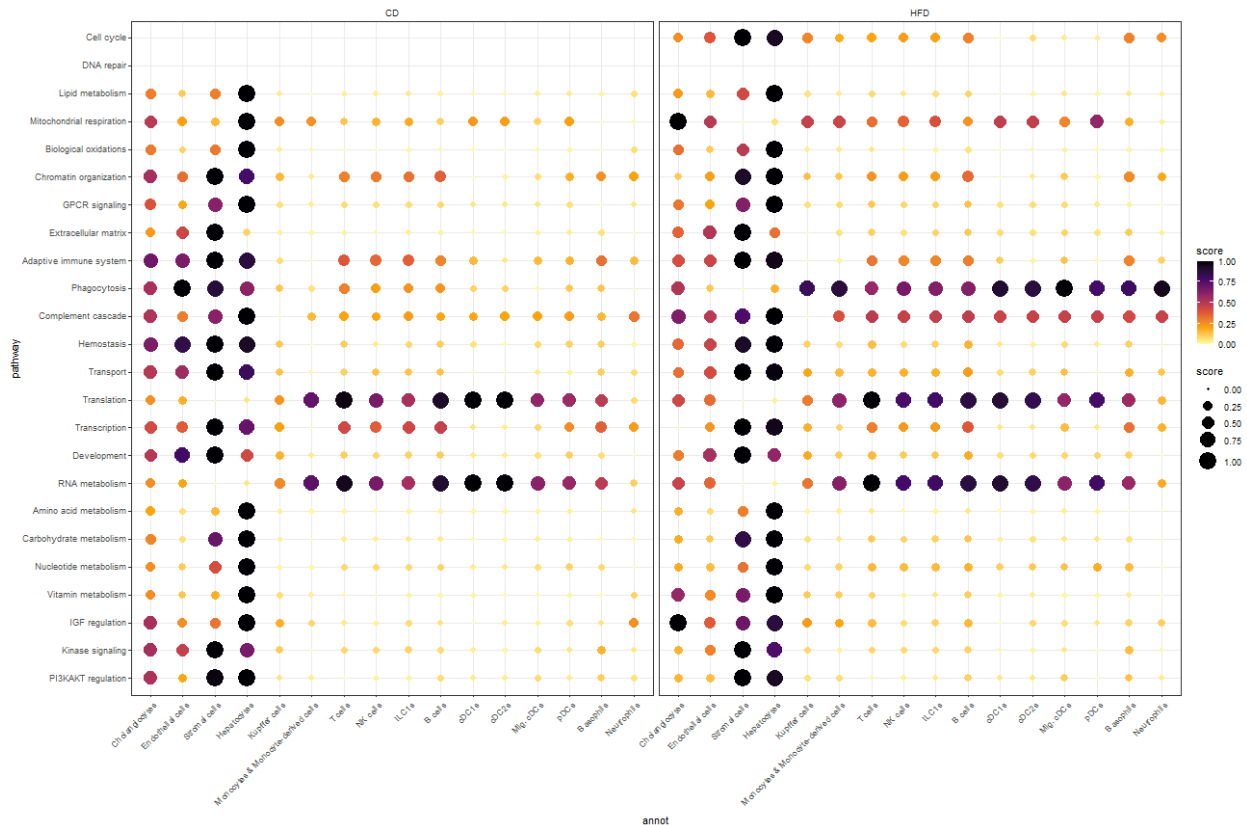


```r
# Cell type enrichment change (CD - HFD)
df4 <- df %>%
```

```
  dplyr::mutate(score = score - df2$score) %>%
  dplyr::mutate(size = abs(score))

ggplot(df4, aes(x=annot, y=pathway, size=score, color=score)) +
  geom_point() +
  scale_color_gradientn(colours = colPals$RdBu) +
  scale_size(range = c(1,8), breaks = c(0,0.5,1), limits = c(0,1))+
  theme_bw() +
  theme(strip.background = element_blank(),
        axis.text.x = element_text(angle = 45, hjust = 1, vjust = 1),
        axis.text.y = element_text(hjust = 1, vjust = 0.3))
```
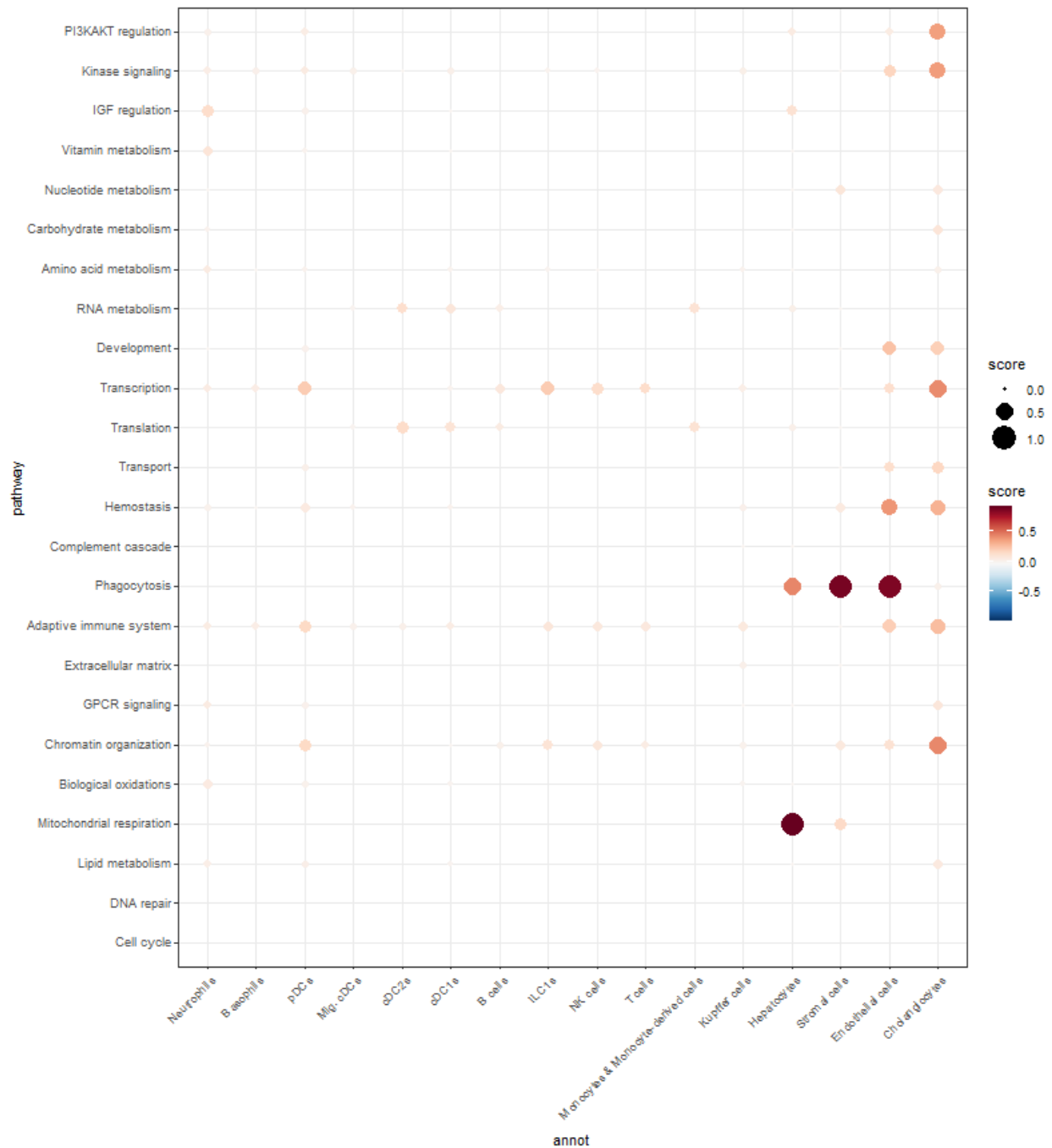
Pathway zonation in the liver

```r
# subsample single-cell data to 5000 cells per cell type for enrichment analysis
dat <- liver$mouseStSt$Visium
subs <- dat@meta.data %>%
  tibble::rownames_to_column(var = 'cellid') %>%
  dplyr::left_join(liver_meta, by = 'sample') %>%
  dplyr::filter(gender == 'male') %>%
  dplyr::group_by(zonationGroup) %>%
```
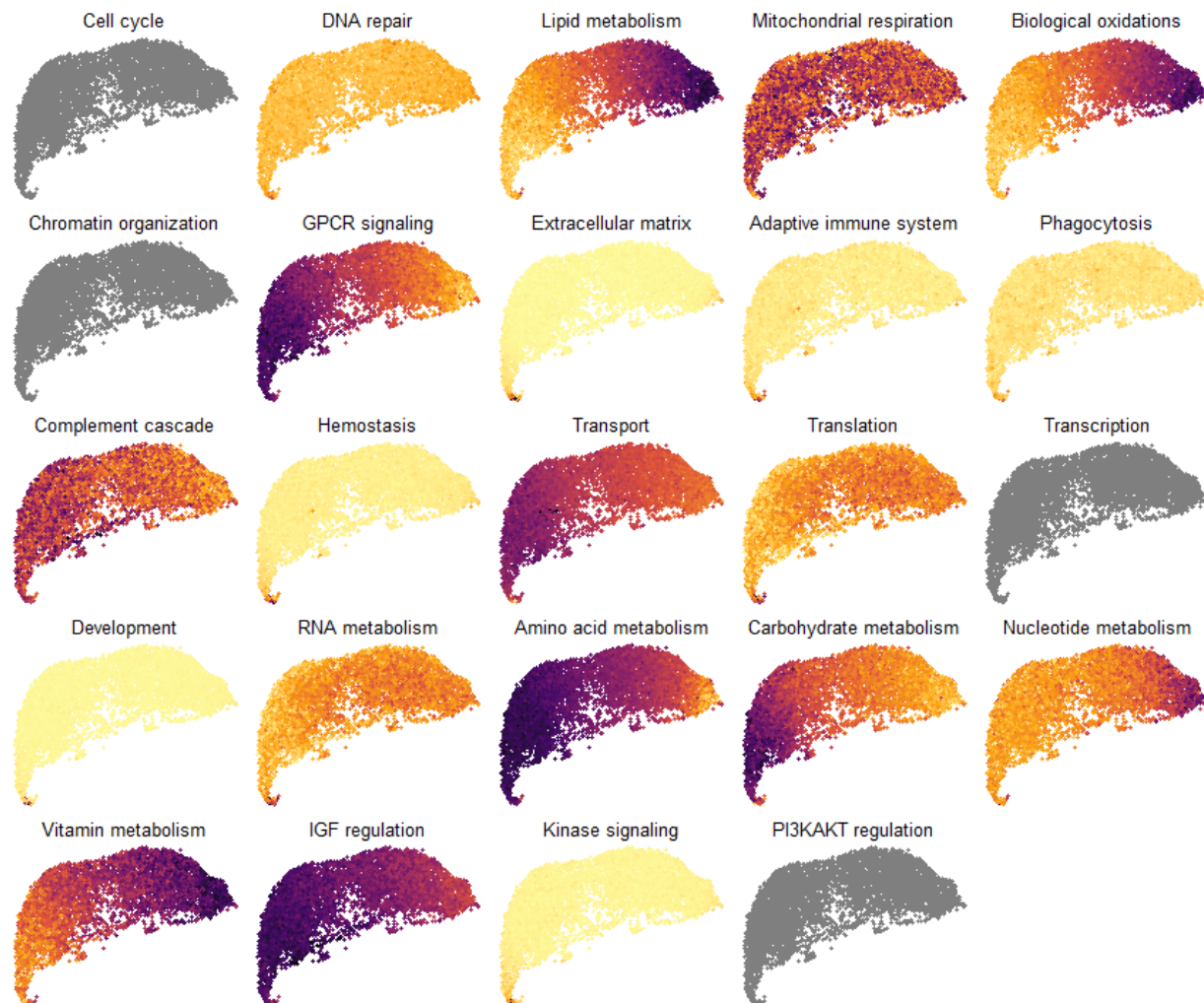
```
    dplyr::slice_sample(n=5000)
dat <- subset(dat, cells = subs$cellid)

res <- getPAS(x = dat,
              gene.sets = pathway_sets,
              npcs = 100)

p <- lapply(names(pathway_sets), function(set) {
  local({
    df <- data.frame(UMAP_1 = res$UMAP_1,
                     UMAP_2 = res$UMAP_2)
    df$score <- res[, set]
    p1 <- ggplot(df, aes(x=UMAP_1, y=UMAP_2, color=score)) +
      geom_point(shape=19, size=1) +
      scale_color_gradientn(colours = colPals$inferno) +
      theme_void() +
      theme(legend.position = 'none',
            plot.title = element_text(hjust = 0.5)) +
      ggtitle(set)
  })
})

patchwork::wrap_plots(p, ncol=5, byrow=T, guides = 'collect')
```

```
sessionInfo()
```

```
## R version 4.0.5 (2021-03-31)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19044)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_United States.1252
## [2] LC_CTYPE=English_United States.1252
## [3] LC_MONETARY=English_United States.1252
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United States.1252
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
##  [1] patchwork_1.1.1    pagoda2_1.0.2      igraph_1.2.6       Matrix_1.3-2
##  [5] SeuratObject_4.0.0 Seurat_4.0.2       forcats_0.5.1      stringr_1.4.0
```

```
##  [9] dplyr_1.0.3           purrr_0.3.4          readr_1.4.0          tidyr_1.2.0
## [13] tibble_3.1.4          ggplot2_3.3.3        tidyverse_1.3.0
##
## loaded via a namespace (and not attached):
##   [1] utf8_1.1.4           reticulate_1.18     R.utils_2.10.1
##   [4] tidyselect_1.1.0     htmlwidgets_1.5.3   grid_4.0.5
##   [7] BiocParallel_1.22.0  Rtsne_0.15          munsell_0.5.0
##  [10] codetools_0.2-18     ica_1.0-2           future_1.21.0
##  [13] miniUI_0.1.1.1       withr_2.4.1         spatstat.random_2.2-0
##  [16] colorspace_2.0-0     Biobase_2.48.0      highr_0.8
##  [19] knitr_1.31           rstudioapi_0.13     stats4_4.0.5
##  [22] ROCR_1.0-11          tensor_1.5          distillery_1.2
##  [25] listenv_0.8.0        labeling_0.4.2      urltools_1.7.3
##  [28] polyclip_1.10-0      farver_2.0.3        extRemes_2.1
##  [31] parallelly_1.23.0    vctrs_0.3.8         generics_0.1.2
##  [34] xfun_0.31            fastcluster_1.1.25  R6_2.5.0
##  [37] locfit_1.5-9.4       flexmix_2.3-17      spatstat.utils_2.3-0
##  [40] RcppArmadillo_0.10.1.2.2 assertthat_0.2.1 promises_1.1.1
##  [43] scales_1.1.1         nnet_7.3-15         gtable_0.3.0
##  [46] Cairo_1.5-12.2       globals_0.14.0      conquer_1.0.2
##  [49] goftest_1.2-2        drat_0.1.8          rlang_0.4.10
##  [52] MatrixModels_0.4-1   splines_4.0.5       lazyeval_0.2.2
##  [55] spatstat.geom_2.4-0  broom_0.7.4         brew_1.0-6
##  [58] yaml_2.2.1           reshape2_1.4.4      abind_1.4-5
##  [61] modelr_0.1.8         backports_1.2.1     httpuv_1.5.5
##  [64] tools_4.0.5          sccore_0.1.2        ellipsis_0.3.2
##  [67] spatstat.core_2.4-2  RColorBrewer_1.1-2  BiocGenerics_0.34.0
##  [70] ggridges_0.5.3       Rcpp_1.0.7          plyr_1.8.6
##  [73] dendsort_0.3.3       rpart_4.1-15        deldir_1.0-6
##  [76] pbapply_1.4-3        cowplot_1.1.1       scde_2.16.0
##  [79] zoo_1.8-8            haven_2.3.1         ggrepel_0.9.1
##  [82] cluster_2.1.1        fs_1.5.0            magrittr_2.0.1
##  [85] data.table_1.13.6    scattermore_0.7     SparseM_1.78
##  [88] p2data_1.0.0         lmtest_0.9-38       triebeard_0.3.0
##  [91] reprex_1.0.0         RANN_2.6.1          pcaMethods_1.80.0
##  [94] fitdistrplus_1.1-3   matrixStats_0.58.0  hms_1.0.0
##  [97] mime_0.9             evaluate_0.14       xtable_1.8-4
## [100] RMTstat_0.3          N2R_0.1.1           readxl_1.3.1
## [103] gridExtra_2.3        compiler_4.0.5      KernSmooth_2.23-18
## [106] crayon_1.4.0         R.oo_1.24.0         htmltools_0.5.2
## [109] mgcv_1.8-33          later_1.1.0.1       lubridate_1.7.9.2
## [112] DBI_1.1.1            dbplyr_2.0.0        MASS_7.3-53
## [115] cli_2.3.0            R.methodsS3_1.8.1   parallel_4.0.5
## [118] pkgconfig_2.0.3      plotly_4.9.3        spatstat.sparse_2.0-0
## [121] xml2_1.3.2           rvest_0.3.6         digest_0.6.27
## [124] sctransform_0.3.2    RcppAnnoy_0.0.19    spatstat.data_2.2-0
## [127] rmarkdown_2.14       cellranger_1.1.0    leiden_0.3.7
## [130] Rook_1.1-1           uwot_0.1.11         edgeR_3.30.3
## [133] shiny_1.6.0          Lmoments_1.3-1      quantreg_5.83
## [136] modeltools_0.2-23    rjson_0.2.20        lifecycle_0.2.0
## [139] nlme_3.1-151         jsonlite_1.7.2      viridisLite_0.3.0
## [142] limma_3.44.3         fansi_0.4.2         pillar_1.6.2
## [145] lattice_0.20-41      fastmap_1.1.0       httr_1.4.2
## [148] survival_3.2-7       glue_1.4.2          png_0.1-7
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## [151] stringi_1.5.3           irlba_2.3.3            future.apply_1.7.0
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